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Evaluation of Effects of Oral Exposure to Ametryn on Development of Mice

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Abstract: The developmental toxicity potential of ametryn was assessed, involving teratogenic and reproductive studies on 180 and 170 presumed pregnant albino mice respectively, given doses of 0, 292, 438, 584 and 779 mg kg^{-1} day^{-1}, orally on days 6–15 of presumed gestation. During the gestation period, body weight, food consumption, water intake, mortality and general behavioural changes were recorded. On day 18, mice for teratogenic studies were killed and some parameters assessed. There were more deaths for the 584 mg kg^{-1} day^{-1} (31%) and 779 mg kg^{-1} day^{-1} (49%) groups than for the 438 mg kg^{-1} day^{-1} (10%) and 292 mg kg^{-1} day^{-1} (0%) groups. Reductions in mean body weight gain during exposure and post-exposure periods were recorded for the two highest dose levels; however, the corrected maternal weight (minus the uterus) remained unchanged for all the groups. Differences in food consumption and water intake were insignificant at all dose levels. Teratogenic parameters such as litter size decreased at the two highest doses as a result of significant resorptions and abortions. Other parameters such as termed fetuses per litter, fetal body weight, placental weight, crown–rump length and tail length, were reduced significantly. No external, visceral or skeletal changes were observed except delayed ossification. These results show that ametryn is embryotoxic to mice at 584 mg kg^{-1} and above. The 170 presumed pregnant mice (F_0) allowed to deliver F_1 pups did so after 20($\pm$2) days and F_2 pups obtained from matured F_1 (not given any ametryn) were also delivered after 20($\pm$1) days.

There was F_1 pup weight reduction for the two highest doses whereas F_2 pups showed a non-significant reduction only for 779 mg kg^{-1} day^{-1} group. F_1 fetal viability was 50–75% before day 4 and 75–99% after day 4 for the two highest doses compared to 100% survival for other dosage groups. No deaths were recorded for F_2 generation pups. Food and water intakes, crown–rump length and tail length increments were insignificant for both generations. The appearance of developmental milestones like pinna attachment, hair growth, vaginal opening and testes descension remained unaffected for all the doses, but times of incisor eruptions, eye and ear opening were slightly lengthened for F_1 generation at 779 mg kg^{-1}. This observation was not noted in F_2 generation pups. A battery of behavioural tests conducted for F_1 and F_2 pups did not reveal changes in movements such as pivoting, negative geotaxis reflex nor in post-weaning test such as consummatory activity and activity wheel. The growth of the skeletal system was unaffected by ametryn after day 10 post-delivery (F_1 generation) and day 0 (F_2) generation. Ametryn has little or no effect on reproductive and/or developmental characteristics of mice at doses below maternal toxicity. © 1998 SCI

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1 INTRODUCTION

Ametryn (N^2-ethyl-N^4-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine; G34 162) is an *s*-triazine herbicide used especially in sugarcane, banana, citrus, pineapple, coffee, tea and other crops either singly or in combination with other herbicides. Less research has been done on it than on its structural analogue atrazine. When it was given to mice at doses of 0, 292, 438, 584 and 779 mg kg^{-1} for five consecutive days, it was shown to be non-mutagenic to mouse spermatogenesis.[1] In a previous study, rats of both sexes fed with 0, 20, 200 or 2000 mg kg^{-1} of ametryn in the diet for two generations showed reductions in body weight, food consumption and water intake, in the highest dose groups, but no external abnormalities were recorded.[2] The absence of these abnormalities in rats has been corroborated. In that work, no fetal abnormalities were seen in fetuses of pregnant rats given ametryn orally at 0, 101, 202, 404 and 539 mg kg^{-1} day^{-1} on days 6–15 of gestation.[3] Nevertheless, ametryn (30·6 mg kg^{-1}, 1/50 LD_{50}) given *in utero* to Wistar albino rats on days 5–15 of gestation was shown to cause a decrease in fetal body weight (5·8%) and an increased incidence of malformed fetuses (29·4% per litter).[4]

This paper describes the teratogenic and reproductive endpoints in mice following treatment with ametryn during the major period of organogenesis.

2 EXPERIMENTAL METHODS

2.1 Chemical

Ametryn 500 g kg^{-1} FW formulation ('Gesapax' 500 FW; A-39529G; P.806054, June 1988) was supplied by Swiss-Nigeria Chemical Company, Lagos.

2.2 Animals

Three hundred and fifty healthy female albino mice (13 weeks old and sexually mature) and 50 male mice, weighing 20·46($\pm$1·79) g and 20·15($\pm$2·12) g, respectively, were used on the day of mating. They were acclimatized to laboratory conditions for two weeks. Each mouse was identified by its cage and groups, and individually by a metal ear tag with a unique number used during the study.

2.3 Teratogenic studies

Each dose group had 30 female mice except the last two highest doses which had 45 mice each. Thirty-five male mice were used to mate 210 females in a ratio of 1 male : 2 females per cage. The process of determining the oestrus cycle was avoided. Each male was introduced into a cage of two virgin females (1800 h) and sperm plug was checked for the next day at 0700 h. The male mice were removed if no sperm plug was seen and used again but not necessarily with the same females. The process was continued until day 0 of gestation (the day sperm plug was seen) was obtained for all the female mice. The mated animals were then assigned to one of the five treatment groups (0, 292, 438, 584 and 779 mg kg^{-1} day^{-1}) using random numbers. From day 6 to day 15 of gestation, each mouse received the appropriate dose of formulated ametryn (without dilution) orally by gavage while the control animals received distilled water. Prior to and after the administration of the test chemical, the animals were observed daily for mortality and unusual clinical and/or behavioural changes. Body weight, food consumption and water intake were recorded daily for the pre-treatment (days 0–5), treatment (days 6–15) and post-treatment (days 16–18) periods.

The mice were killed by carbon dioxide asphyxiation on day 18 of gestation. The uteri of the animals were excised, blotted and weighed. The number of implantations, resorption (both early and late), corpora lutea, and the number and position of viable and non-viable male and female fetuses were recorded. Also recorded were number of abortions, fetal body weight, placental weight, tail length, crown–rump length and sex ratio. Uteri of non-pregnant females were immersed in 10% ammonium sulfide for 10 min and examined for dark residues corresponding to implantation sites, and recorded as early resorptions. The organ weights (heart, lungs, spleen, liver and kidneys) of dams were also recorded.

Live fetuses were examined for external gross abnormalities such as cleft lip and cleft palate. Half of the fetuses were fixed in Bouin's solution for visceral or soft tissue examination, including renal pelvis size and hydrocele of the ureter. The other half were fixed in 95% ethanol, cleared and stained with 2% KOH with Alizarin red S (1 mg dl^{-1}) on the first day and 1% KOH with Alizarin red S (1 mg dl^{-1}) the second day for skeletal anomalies such as the presence of supernumerary ribs, and ossification of bones.[5] The examinations were done with the aid of a dissecting microscope.

2.4 Reproductive studies

A total of 170 presumed pregnant mice (F_0) were used in this work. The control, 292 and 438 mg kg^{-1} day^{-1} groups had 30 mice each, compared to 40 mice for the two highest groups. Each mouse received appropriate doses of formulated ametryn (without dilution) orally by gavage on days 6–15 and were allowed to deliver and wean their litters. Daily maternal mortality, body

weight, changes in food consumption and water intake were recorded from day 0 to the day prior to delivery. After delivery, fetal parameters (F_1) measured included litter size, sex, daily fetal weight, tail length, crown–rump length for 60 days, with addition of food consumption and water intake during weaning period (day 21 after birth).

The appearance of some developmental landmarks such as eyes and ears opening, hair growth, incisors eruption, testes descension and vaginal opening were assessed. Skeletal development of six mice per dose was evaluated on days 0, 10, 20 and 50 post-delivery. This was done by removing the skins of day 10, 20 and 50 mice and placing them in 10% aqueous KOH with Alizarin red S (1 mg dl^{-1}) until the desired degree of staining was obtained.[5] The numbers of unossified and malformed bones were compared to those of the control.

Pre-weaning behavioural tests were performed[6] (a) pivoting locomotion (time taken for a mouse to move within four quadrants of a 9-cm (diameter) circle, days 5–14; (b) negative geotaxis reflex (time required to orient 180° uphill), days 2–15; (c) surface righting reflex (time needed to rise from a supine to a prone position), days 1–10; (d) air righting reflex (free-fall from 30 cm above a flat surface), days 1–10; (e) visual placing reflex (reaching for a surface when held suspended above it), days 14–25. Post-weaning behavioural tests conducted were consummatory behaviour which included food and water intakes. This was determined by the quantity of food or water devoured in three consecutive days within day 30 and 60 post-delivery. Activity wheel[6] involved placing 50-day-old mice on a rotatory rod (diameter 2·5 cm) at 10 revolution per min. At 30 and 60 s, the speed was increased to 20 and 30 rev min^{-1} respectively. The length of time that the mice remained on the rod in two trials was recorded.

Five groups of 20 mature F_1 female mice each, age 60–65 days, were mated with 25 F_1 mature males as earlier described. Mating was done between mice of different litters but of the same dosage groups, although ametryn was not administered to them. The parameters determined for F_1 generation, such as maternal daily mortality and body weight were determined from day 0 of gestation to delivery. The fetal assessment included litter size, sex, daily pup weight, tail length and crown–rump length for 50 days. On day 21 the pups were weaned, and daily food consumption and water intake for 50 days were recorded. The appearance of developmental landmarks, pre-weaning and post-weaning behavioural tests, as well as skeletal and soft tissue analysis were performed.

2.5 Statistics

Sex ratio was analysed for statistical significance using binomial distribution test. Survival indices and abnormalities like skeletal morphological and visceral abnormalities of the neonates were analysed using the Mann–Whitney U test. Any other parameter was subjected to ANOVA (one-way) with significant values subjected again to Student's *t*-test. All effects were reported significant at $P < 0{\cdot}05$.

3 RESULTS

3.1 Teratogenic studies

3.1.1 Maternal effects. There were no mortalities among the 0 and 292 mg kg^{-1} day^{-1} groups, compared to 438 mg kg^{-1} day^{-1} (10%), 3 out of 30 dams; 584 mg kg^{-1} day^{-1} (31%), 14 out of the 45 dams; and 779 mg kg^{-1} day^{-1} (49%), 22 out of the 45 dams. These deaths occurred primarily within the first two days of treatment. Treatment-related clinical signs (dyspnea, reduced skeletal muscular movements, ataxia and salivation) were also observed in the two highest dose groups during treatment. Food consumption and water intake were not different from the control. Absolute body weight gain for the entire period of study (days 0–18) and treatment period (days 6–15) was reduced for animals in the two highest dose groups, but not for the 292 and 438 mg kg^{-1} day^{-1} groups. Uterine weights were significantly lowered at 584 and 779 mg kg^{-1} day^{-1}; corrected body weight gain (terminal weight minus gravid uterus) was not different among the groups. Absolute and relative weights of the heart, lungs, spleen, liver and kidneys were not different from the control (Table 1).

3.1.2 Fetal effects

There were 24 pregnant dams out of 30 in the control and 292 mg kg^{-1} day^{-1} groups, 22 out of 30 in the 438, 28 out of 45 in the 548 and 23 out of 45 in the 779 mg kg^{-1} day^{-1} groups. There were no abortions in the 0, 292 and 438 mg kg^{-1} day^{-1} groups but 5 complete abortions occurred in the 584 and 8 in the 779 mg kg^{-1} day^{-1} groups in days 14 to 17 of gestation. The number of implantations and corpora lutea per animal did not differ from the control, but the number of resorptions per dam was significantly increased at the two highest doses. Mean total fetuses and viable fetuses, fetal body weight, placental weight, tail length and crown–rump length were reduced significantly for the two highest doses. Fetal sex ratios were unaffected (Table 2).

Examination of the placentae revealed the presence of whitish particles located at the periphery in treated mice; the quantity of these particles increased with dosage. No external morphological effects were seen in any of the fetuses, nor were there any soft tissue, visceral

TABLE 1
Maternal Toxicity Data Obtained from Mice Exposed to Ametryn during Organogenesis for Teratogenic Studies

Parameter[a,b]	*Dose (mg kg^{-1} day^{-1})*				
	0	*292*	*438*	*584*	*779*
Dams (no.)	30	30	30	45	45
No. dead (%)	0	0	3 (10)	14 (31)	22 (49)
No. pregnant & alive (%)	24 (80)	24 (80)	22 (73)	28 (62)	23 (51)
Food consumption (g kg^{-1} day^{-1})	354·8 (±41·4)	342·8 (±39·1)	311·4 (±40·9)	290·3 (±84·6)	376·3 (±34·3)
Water intake (ml kg^{-1} day^{-1})	374·1 (±38·6)	354·2 (±41·1)	399·5 (±29·8)	332·4 (±36·7)	361·6 (±49·4)
Maternal weight gain (g):					
Day 6–15	5·7 (±1·8)	5·8 (±1·5)	4·8 (±1·9)	3·9 (±1·0)*	3·4 (±1·2)**
Day 0–18	9·4 (±1·3)	9·0 (±1·1)	8·9 (±0·9)	6·3 (±0·8)*	5·7 (±1·3)**
Uterine wt (g)	8·4 (±1·3)	7·9 (±1·5)	7·1 (±1·4)	5·9 (±2·3)*	4·8 (±1·8)**
Corrected body weight[c] (g)	17·4 (±1·6)	18·2 (±2·1)	18·6 (±2·7)	17·2 (±2·2)	17·3 (±2·4)
Organ weight[d] (g):					
Liver (ab.)	0·89 (±0·28)	0·91 (±0·10)	1·11 (±0·12)	1·01 (±0·12)	1·01 (±0·10)
(rel.)	46·3 (±7·8)	48·7 (±4·3)	47·1 (±6·3)	49·9 (±8·4)	48·6 (±9·8)
Spleen (ab.)	0·11 (±0·02)	0·11 (±0·03)	0·11 (±0·03)	0·09 (±0·01)	0·09 (±0·02)
(rel.)	7·14 (±2·56)	7·21 (±2·89)	7·29 (±2·42)	8·19 (±2·39)	8·89 (±1·95)
Heart (ab.)	0·09 (±0·03)	0·09 (±0·02)	0·09 (±0·02)	0·09 (±0·01)	0·09 (±0·01)
(rel.)	4·04 (±0·48)	4·01 (±0·44)	4·20 (±0·46)	4·62 (±0·06)	4·58 (±0·66)
Lungs (ab.)	0·16 (±0·02)	0·16 (±0·03)	0·16 (±0·02)	0·16 (±0·03)	0·16 (±0·03)
(rel.)	7·26 (±1·31)	7·11 (±0·80)	7·08 (±0·91)	7·09 (±0·72)	7·09 (±0·87)
Kidneys (ab.)	0·10 (±0·03)	0·11 (±0·01)	0·11 (±0·01)	0·11 (±0·02)	0·12 (±0·02)
(rel.)	4·89 (±0·77)	5·46 (±1·25)	5·46 (±1·25)	5·27 (±0·64)	6·36 (±1·84)

[a] Values expressed as mean (±SD).
[b] Significant difference from control at * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.
[c] Corrected weight = absolute maternal body weight − uterine weight.
[d] ab. = absolute; rel. = relative.

TABLE 2
Effect of Exposure of Fetuses to Ametryn during Organogenesis, Day 6–15

Parameter[a,b]	*Dose (mg kg^{-1} day^{-1})*				
	0	*292*	*438*	*584*	*779*
Pregnant dams (no.)	24	24	22	28	23
Abortions[c] (no.)	0	0	0	5	8
Implantation/preg.dam	10·3 (±0·9)	10·6 (±0·8)	10·6 (±0·6)	10·9 (±0·5)	10·8 (±0·1)
Corpora lutea/preg dam	11·1 (±0·6)	10·9 (±0·8)	11·1 (±0·8)	10·8 (±0·9)	10·9 (±0·9)
Fetuses/preg.dam	9·9 (±1·8)	10·0 (±1·9)	10·1 (±1·6)	7·8 (±1·9)*	6·5 (±1·7)**
Resorption/preg.dam	1·3 (±0·6)	1·1 (±0·5)	1·2 (±0·7)	4·1 (±1·0)**	5·0 (±1·8)**
Fetal body weight (g)	1·48 (±0·71)	1·46 (±0·82)	1·42 (±0·66)	1·24 (±0·14)*	1·09 (±1·18)**
Placental weight (g)	0·14 (±0·01)	0·13 (±0·01)	0·14 (±0·01)	0·10 (±0·01)**	0·10 (±0·02)**
Tail length (cm)	1·28 (±0·15)	1·29 (±0·18)	1·29 (±0·13)	0·9 (±0·30)**	0·7 (±0·44)**
Crown–rump length (cm)	2·81 (±0·22)	2·93 (±0·31)	2·71 (±0·19)	2·13 (±0·31)**	2·04 (±0·20)**
Fetal sex ratio (M : F)	1 : 1	1 : 1	0·9 : 1·1	1·1 : 0·9	1 : 1

[a] Values expressed as mean (±SD).
[b] Significant difference from control at * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.
[c] Number of mice which had complete abortion.

TABLE 3
Skeletal Examination of Fetuses Obtained from Ametryn-Treated Mice during Teratogenic Studies

Parameter	*Dose (mg kg^{-1} day^{-1})*				
	0	*292*	*438*	*584*	*779*
No. fetuses/litter examined	90/16	90/17	90/17	100/16	80/15
No. bones affected/litter:					
Cranium:					
Inter parietal bones poorly ossified	0	0	0	9/10	50/14
Larger anterior fontanelle opening	0	0	0	11/12	47/11
Larger posterior fontanelle opening	0	0	0	14/11	52/18
Thoracic cage:					
Less than 13 full pairs of ribs	0	0	0	0	0
Unossified sternabrae	0	0	0	0	0
No. of vertebrae per fetus:					
Cervical	7	7	7	7	7
Thoracic	13	13	13	13	13
Lumbar	6	6	6	6	6
Sacral	4	4	4	4	4
Coccygeal	5–6	5–6	5–6	3–5	1–4
No. of forelimbs per fetus					
Metacarpals	4	4	4	4	4
Proximal	4	4	4	2–4	2–3
Middle	4	4	4	2–4	2–3
Distal	4	4	4	2–4	2–3
No. of hind-limbs					
Metatarsals	5	5	5	5	4–5
Proximal	5	5	5	5	4–5
Middle	4	4	4	4	3–4
Distal	5	5	5	4–5	3–4

No skeletal abnormality was seen except reduction of ossification noticed in some bones, mainly the fore- and hind-limbs, coccygeal and cranium.

TABLE 4
Reproductive Indices of Ametryn-Treated Mice (F_0) Generation

Parameter	*Dose (mg kg^{-1} day^{-1})*				
	0	*292*	*438*	*584*	*779*
No. of dams	30	30	30	45	40
No. of pregnant mice (%)	25 (83)	30 (100)	29 (97)	35 (87)	20 (50)
Duration of gestation (days)	18–20	18–20	18–20	18–21	18–21
Dam weight[a,b] *(g)*:					
Gestational days					
0–3	20·2 (±0·9)	20·3 (±1·4)	20·0 (±0·5)	22·0 (±2·8)	20·9 (±2·6)
4–6	20·7 (±1·0)	20·9 (±2·3)	20·5 (±1·6)	23·0 (±3·3)	20·1 (±1·9)
7–9	21·7 (±1·3)	21·9 (±2·7)	21·8 (±0·8)	25·5 (±3·7)	21·6 (±3·0)
10–12	24·0 (±3·2)	23·8 (±3·1)	23·7 (±1·1)	27·2 (±4·8)	22·7 (±2·6)
13–15	27·9 (±3·9)	27·8 (±4·7)	27·6 (±2·3)	30·0 (±5·1)	25·4 (±3·9)
16–18	31·8 (±4·0)	32·2 (±4·2)	31·2 (±2·0)	31·2 (±5·7)	29·0 (±5·0)*
19–21	23·6 (±4·5)	34·1 (±4·3)	33·8 (±4·5)	30·4 (±6·1)*	29·1 (±6·6)*
Average weight gain (day 0–21)	13·2 (±2·3)	14·08 (±3·2)	13·8 (±3·7)	8·4 (±4·2)*	8·2 (±4·5)*

[a] Values expressed as mean (±SD).
[b] Significant difference from control at * $P < 0{\cdot}05$.

or skeletal abnormalities present. However, bones of the extremities, skull and coccygeal showed delayed ossification (Table 3).

3.2 Reproduction

Mice (F_0) in the 584 and 779 mg kg^{-1} day^{-1} groups had slightly longer gestation periods than those in the other groups, but the difference was not sufficient to conclude that there was a delay in implantation. Maternal body weights were slightly reduced during the post-treatment period (days 16–18) for the 779 mg kg^{-1} day^{-1} group (Table 4) and days 19–21 for the two highest doses. The number of abortions was increased at the two highest doses and the litter size reduced significantly for the 779 mg kg^{-1} day^{-1} dose group. Pup sex-ratios were not different between the control and the treated groups (Table 5).

At birth of F_1, there was a reduction in the pup weights for the first five days in the highest dose group, and, on day 0, for tail length and crown–rump length in the 779 mg kg^{-1} day^{-1} group but no difference in the daily body weight, tail length and crown–rump increment between the treatment and control groups was observed. The F_1 percentage daily pup-weight increment decreased during growth but remained lower in value for 779 mg kg^{-1} day^{-1} compared to other treatment groups. F_1 pups in ametryn-treated groups did not differ from the control in terms of external observations. One high dose (779 mg kg^{-1} day^{-1}) pup had a swollen abdomen filled with fluid two days post-partum and died the next day, but this was not considered to be related to treatment. There was decreased pup viability before day 4 at the two highest doses and

TABLE 5
Evaluation of F_1 Pups' Development Following Their Delivery from Ametryn-Treated Mice

Parameter[a,b]	*Dose (mg kg^{-1} day^{-1})*				
	0	*292*	*438*	*584*	*779*
No. delivered/dam	9·1 (±1·2)	9·5 (±1·3)	9·2 (±1·5)	7·4 (±2·6)*	6·4 (±1·6)**
% Abortion/dam	0	0	0	30 ± 10	40 ± 5
Sex ratio (M : F)	1 : 1	0·9 : 1·1	1 : 1	0·9 : 1·1	0·9 : 1·1
Viability before day 4 (%)	100	100	100	70 (±5)	60 (±10)
Viability after day 4 (%)	100	100	100	91·8 (±8·1)	86·7 (±10·7)
Pup weight (g):					
Day					
0	1·35 (±0·09)	1·38 (±0·07)	1·43 (±0·07)	1·23 (±0·12)	1·05 (±0·13)*
5	2·57 (±0·42)	2·52 (±0·28)	2·83 (±0·16)	2·58 (±0·53)	2·01 (±0·12)*
20	7·03 (±0·42)	7·08 (±0·79)	7·44 (±0·95)	6·95 (±1·15)	6·42 (±0·50)
50	18·83 (±2·14)	18·71 (±1·65)	18·40 (±1·96)	17·49 (±1·86)	17·31 (±2·55)
Crown–rump & tail:[c]					
Length (cm):					
0	2·49 (±0·11)	2·42 (±0·16)	2·39 (±0·19)	2·04 (±0·15)	1·88 (±0·07)*
	[1·26 (±0·08)]	[1·23 (±0·09)]	[1·24 (±0·05)]	[1·18 (±0·06)]	[1·16 (±0·08)]*
20	5·20 (±0·13)	5·15 (±0·16)	4·95 (±0·34)	4·49 (±0·15)	4·62 (±0·19)
	[5·96 (±0·15)]	[5·99 (±0·19)]	[5·80 (±0·19)]	[5·68 (±0·46)]	[5·25 (±0·30)]
50	7·09 (±0·16)	7·19 (±0·21)	7·11 (±0·13)	7·18 (±0·32)	7·12 (±0·41)
	[8·53 (±0·41)]	[8·63 (±0·43)]	[8·58 (±0·23)]	[8·56 (±0·44)]	[8·52 (±0·34)]
Food and water[c] (*g or ml kg^{-1} day^{-1}*):					
Day					
21	343 (±95)	379 (±115)	370 (±87)	361 (±81)	373 (±93)
	[412 (±107)]	[394 (±98)]	[391 (±75)]	[389 (±114)]	[401 (±109)]
30	502 (±64)	489 (±93)	508 (±99)	492 (±93)	496 (±87)
	[652 (±94)]	[564 (±101)]	[639 (±97)]	[589 (±69)]	[549 (±89)]
50	395 (±94)	387 (±93)	366 (±87)	402 (±89)	392 (±79)
	[384 (±98)]	[413 (±99)]	[392 (±85)]	[398 (±103)]	[401 (±89)]

[a] Values are expressed as mean (±SD).
[b] Significant difference from control at * $P < 0{\cdot}05$.
[c] Tail length and water consumption are in brackets.

also after day 4 in the 779 mg kg^{-1} day^{-1} group. Post-weaning food and water consumption did not differ significantly from the control (Table 5). In the 779 mg kg^{-1} day^{-1} pups, there was a slight delay (not more than one day) in the appearance of several developmental landmarks (eye opening, ear opening, incisor eruption, testes descension) (Table 6). There were no differences among the groups in any of the pre-weaning or post-weaning behavioural tests. The possibility of delayed ossification in the 584 and 779 mg kg^{-1} day^{-1}

TABLE 6
Days of Appearance of Development Landmarks

	Dose (mg kg^{-1} day^{-1})				
Parameter	*0*	*292*	*438*	*584*	*779*
Pinna attachment	4	4	4	4–5	4–5
Hair growth	4–5	4–5	4–5	4–5	4–5
Incisor eruption	10–11	10–11	10–11	10–11	10–12
Ear opening	12–15	12–14	12–15	13–15	13–16
Eye opening	13–15	12–14	12–14	12–15	14–16
Testes descension	23–25	23–25	23–25	24–25	24–26
Vaginal opening	24–26	25–26	25–26	25–26	25–26

[a] Pups in 779 mg kg^{-1} day^{-1} group had slight extension in the period of appearance.

TABLE 7
Parameters of F_1 Generation Dams and F_2 Generation Pups

	Dose (mg kg^{-1} day^{-1})				
Parameter	*0*	*292*	*438*	*584*	*779*
No. dams	20	20	20	20	20
No. pregnant (%)	18 (±90)	17 (±95)	19 (±95)	19 (±95)	16 (±80)
Food consumption (g kg^{-1} day^{-1})	389·4 (±87·1)	372·4 (±81·3)	353·7 (±99·9)	368·1 (±74·8)	364·8 (±73·4)
Water intake (ml kg^{-1} day^{-1})	364·2 (±66·7)	312·8 (±53·2)	331·7 (±72·1)	364·0 (±71·4)	339·0 (±84·3)
Maternal weight[a] *(g)*					
Day 0	20·4 (±1·8)	19·7 (±1·4)	20·1 (±1·6)	19·8 (±1·1)	18·7 (±1·2)
Day prior to delivery	33·2 (±4·2)	32·4 (±3·6)	33·6 (±4·6)	31·6 (±4·1)	30·6 (±3·9)
Maternal weight gain[a] *(g)*:					
Day 0–18	9·1 (±1·6)	9·4 (±1·5)	9·2 (±1·3)	9·1 (±1·4)	8·8 (±1·1)
Uterine weight	8·2 (±1·4)	8·5 (±1·9)	8·0 (±1·6)	8·1 (±1·5)	7·8 (±1·6)
Duration of gestation (days)	18–20	18–20	18–20	18–20	18–20
Fetal parameters[a]*:*					
Fetuses/pregnant dam	10·2 (±1·8)	10·1 (±1·4)	10·3 (±1·2)	10·2 (±1·3)	9·9 (±1·1)
Fetal body weight (g)	1·39 (±0·9)	1·41 (±0·8)	1·40 (±0·8)	1·39 (±0·7)	1·36 (±0·6)
Placental weight (g)	0·14 (±0·01)	0·14 (±0·01)	0·14 (±0·02)	0·13 (±0·07)	0·13 (±0·06)
Tail length (cm)	1·27 (±0·13)	1·28 (±0·12)	1·26 (±0·14)	1·27 (±0·13)	1·27 (±0·12)
Crown-rump length (cm)	2·42 (±0·40)	2·39 (±0·31)	2·38 (±0·33)	2·39 (±0·38)	2·36 (±0·32)
Fetal sex ratio	1 : 1	1 : 1	1 : 1	1 : 1	0·9 : 1·1
Days of appearance of developmental milestone:					
Pinna attachment	4	4	4	4	4
Hair growth	4–5	4–5	4–5	4–5	4–5
Incisor eruption	10–11	10–11	10–11	10–11	10–11
Eye opening	12–15	12–15	12–15	12–15	12–15
Ear opening	12–15	12–15	12–15	12–15	12–15
Testes descension	23–25	23–25	23–25	23–25	23–25
Vaginal opening	25–26	25–26	25–26	25–26	25–26

[a] Mean values (±SD).

groups suggested by the teratogenic studies (Table 7) was unrecognisable 10 days post-partum.

F_2 generation parameters such as maternal weight, food consumption, water intake, pup weight, crown–rump length, tail length, sex, daily growth increment, developmental milestones such as pinna attachment, hair growth, incisor eruption, ear and eye opening were not influenced by the treatment. However, the reduction in F_1 pup weight continued to F_2 pups in the 779 mg kg^{-1} day^{-1} group (Table 7).

4 DISCUSSION

4.1 Teratogenic studies

This assessment of the effects of ametryn given orally to mice during the period of organogenesis showed that the test material produced significant acute toxicity at doses equal to or greater than 584 mg kg^{-1} day^{-1}. Mortality was markedly higher at these doses and most of the deaths were seen very shortly after the first treatment. The cause of the death was not determined but could be associated with chemically induced toxicity. Toxicity-related clinical signs were apparent shortly after dosing. The reduction in body-weight gain during gestation, and the lack of reduction when gravid uteri weights were subtracted, indicated that the lower weight gain recorded could be a result of reduction in mean litter size and litter weight. This suggests that pregnant mice were more tolerant to treatment with ametryn than are rats.[3] The absence of difference in food and water consumption also supported this possibility.

Although maternal toxicity was not clearly reflected in maternal body weight, fetal toxicity at the 584 and 779 mg kg^{-1} day^{-1} doses seemed to be more pronounced. There was no evidence of embryo or fetotoxicity at dose levels below 438 mg kg^{-1} day^{-1}. The numbers of total fetuses and viable fetuses were significantly reduced at the two highest doses, and the numbers of abortions and resorptions, particularly early resorptions, were significantly increased. Fetal body weights, placental weights, tail length and crown–rump length were also reduced. The evidence of delayed ossification also indicated a retarding effect of maternal treatment with ametryn on the growth phase of gestation. This finding was similar to that of the previous work done with rats.[3] The whitish appearance of the placenta, which increased with ametryn dose, suggested that the latter caused changes in the organ which could be responsible for most of the embryotoxic effects observed.

4.2 Reproductive studies

The objective of these studies was to assess the possible post-natal effects on offspring following maternal exposure to ametryn during the major period of organogenesis for F_1 generation mice. Minor and non-significant differences were found between the offspring of the treated animals and the controls. Although body weights of the pups in the two highest dose groups were slightly lower than controls throughout development, daily body weight increments were not. The slight delays in pups reaching some developmental milestones are of questionable importance. The finding that ametryn did not affect performance on a battery of behavioural tests suggests that only a toxicologically insignificant amount, if any, of the chemical crossed the fetal blood–brain barrier.

F_2 generation pups of 779 mg kg^{-1} day^{-1} groups had slight but non-significant body weight reduction as a continuation of the F_1 generation mothers. All other parameters remained insignificantly different from control.

5 CONCLUSION

From the results of these studies it can be concluded that ametryn had little or no effect on reproductive and/or developmental features of mice at doses below maternal toxicity.

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